COMPOSITION AND METHOD FOR FACILITATING BONE HEALING

The present invention generally relates to nutritional compositions for facilitating bone healing and uses thereof.

Bone is a dynamic living tissue and is continuously being replenished by resorption and deposition of bone matrix. The stability of bone depends upon the underlying connective tissue. Oxlund H. *et al.* has shown that optimally structured collagen is more important for bone strength than bone compactness and its calcium saturation (Bone 1996; 19:479-84). A concentration of cross-links between collagen strands appeared 30% less in bone affected by osteoporosis. Knot L. *et al.* has shown that collagen structure and spatial organization of its fiber network is critical for deposition of minerals and compactness in the bone, and that the micro-architecture of collagen determines bone strength (Bone 1998; 22:181-7). Savvas M. *et al.* has shown that a loss of collagen caused by malnutrition is a major factor in loss of bone mass (J. Obstetr. Gynecol. 1989; 96:1392-4). The anorexic women in the study show a lowered bone density of 18% in the spine and 25% in the femur. The decrease in bone mass is associated with a 22% decrease in skin collagen.

The healing process after bone fracture is an orderly process that involves multiple phases including: i) hematoma formation; ii) fibro-cartilaginous callus formation; iii) bony callus formation; and iv) bone remodeling. During the healing process, pluripotential cells in the vicinity of the bone fracture differentiate into osteoblasts and chondrocytes. Osteoblasts origin form osteoid tissues and they lay down collagen fibers. Chondrocytes give rise to hypertrophic chondrocytes that deposit a mineralized matrix to form calcified cartilage, which is then remodeled into compact bone.

Despite advances in orthopaedic techniques, healing of bone fractures is a lengthy process, often requires weeks if not months. A patient often suffers a severe restriction of movement for several weeks. Facilitating bone healing and fracture repair (i.e., reducing healing time) would be a great relief to the patient. This is particularly desirable in adolescents because this age group of individuals has the lowest compliance with doctor recommendations. When

doctors recommend that an adolescent use crutches for a certain period of time, they often remove themselves from crutches much earlier than recommended.

U.S. Pat. 6,258,778 discloses a method of enhancing bone and cartilage repair by administering angiotensin and its analogues. U.S. Pat. 5,502,074 discloses a method of facilitating bone healing using benzothiophenes. The safety of use of these drugs are not established. For example, angiotensin is known to exert potent cardiovascular and renal effects, and its use in patients with heart or renal failure may be limited.

U.S. Pat. 6,061,597 discloses the application of resonant frequency stimulation to promote fracture healing. U.S. Pat. 6,290,714 discloses a low level laser therapy in treating bone fracture. The effectiveness of these approach is not shown and requires expensive medical office visits and/or computer equipment. None of these methods has been clinically proven.

U.S. Pat. 5,232,709 discloses a nutritional supplement having a large dose of calcium in treating bone loss. Administering to a bone fractured individual with a large dose of calcium would cause mineralization of the bone tissue, rather than supplementing bone collagen. The increased bone mineralization causes further hardening of bone. The affected bone becomes more brittle over time, making it prone to compound fractures and shattering under stress.

There is a long felt need to provide a safe, convenient, affordable and effective approach to facilitate bone healing (i.e., reduce healing time of bone fractures) in humans.

Thus, the technical problem underlying the present invention must be seen as the provision of means and methods to comply with this need. This technical problem is solved by the embodiments characterized in the claims.

Accordingly, the present invention relates to a nutritional composition comprising lysine, proline, ascorbic acid, copper, and vitamin B_6. The nutritional composition is suitable for human use and is effective in facilitating bone healing. The nutritional composition is also suitable for animal use. Preferably, said animal is mammal, most preferably a dog, cat or horse.

Preferably, the nutritional composition contains 27-34 % wt lysine, 14-15 % wt proline, and 42-47 % wt ascorbic acid.

Preferably, the nutritional composition is administered orally. Preferably, the recommended amount is 1,010 mg – 8 gram lysine, 560 mg - 4 gram proline, 1,500 mg – 9 gram ascorbic acid, 2 μg – 6 mg copper, and 0.5 mg – 10 mg vitamin B_6. More preferably, a recommended amount is 230 mg – 10 gram lysine, 120 mg - 5 gram proline, 360 mg – 15 gram ascorbic acid, 1.5 μg – 20 mg copper, and 0.2 mg - 20 mg vitamin B_6. Most preferably, a recommended amount is 1,010 mg lysine, 560 mg proline, 1,500 mg ascorbic acid, 330 μg copper and 10 mg vitamin B_6.

Preferably, the nutritional composition is a daily dosage (based on a human subject of average body weight of 72 kg) of 3.2 – 139 mg/kg lysine, 1.7 – 69.4 mg/kg proline, 5 – 208.3 mg/kg ascorbic acid, 0.02 – 278 μg/kg copper, 2.78 – 279 μg/kg vitamin B_6.

More preferably, the nutritional composition is a daily dosage of 14 – 111 mg/kg lysine, 7.8 – 55.6 mg/kg proline, 20.8 - 125 mg/kg ascorbic acid, 0.03 – 83.3 μg/kg copper, and 6.94 – 139 μg/kg vitamin B_6.

Most preferably, the nutritional composition is a daily dosage of 14 mg/kg lysine, 7.8 mg/kg proline, 20.8 mg/kg ascorbic acid, 4.6 μg/kg copper, 139 μg/kg vitamin B_6.

Preferably, the nutritional composition further comprises vitamin A, vitamin D_3, vitamin E, vitamin B_1, vitamin B_2, niacin, folic acid, vitamin B_{12}, biotin, pantothenic acid, calcium, phosphorus, magnesium, zinc, selenium, manganese, chromium, molybdenum, potassium, citrus fruit peel bioflavanoids, arginine, cysteine, inositol, carnitine, coenzyme Q_{10}, and pycnogenol.

Preferably, the recommended amount is 67 μg -100 mg vitamin A, 0.7 μg - 50 μg vitamin D_3, 0.7 μg - 50 μg vitamin E, 1.4 mg – 8 mg vitamin B_1, 1.4 mg – 8 mg vitamin B_2, 9 mg – 250 mg niacin, 18 μg – 500 μg folic acid, 4 μg – 100 μg vitamin B_{12}, 13 μg - 400 μg biotin, 8 mg – 100 mg pantothenic acid, 7 mg – 40 mg calcium, 3 mg – 300 mg phosphorus, 40 mg – 200 mg magnesium, 0.5 mg – 10 mg zinc, 20 μg - 300 μg selenium, 0.8 mg – 15 mg manganese, 2 μg - 200 μg chromium, 0.8 μg - 100 μg molybdenum, 4 mg – 300 mg potassium, 20 mg – 500 mg citrus fruit peel bioflavanoids, 10 mg – 500 mg arginine, 10 mg – 400 mg cysteine, 5 mg – 400 mg inositol, 5 mg – 400 mg carnitine, 1.6 mg – 70 mg coenzyme Q_{10}, and 1.6 mg – 70 mg pycnogenol.

re preferably, the recommended amount is 166 µg -50 mg vitamin A, 1.65 µg - 20 µg
min D_3, 1.65 µg - 20 µg vitamin E, 3.5 mg – 7 mg vitamin B_1, 3.5 mg – 7 mg vitamin B_2,
5 mg – 100 mg niacin, 45 µg – 300 µg folic acid, 10 µg – 50 µg vitamin B_{12}, 32 µg - 300
biotin, 20 mg – 60 mg pantothenic acid, 17 mg – 35 mg calcium, 7 mg – 100 mg
sphorus, 50 mg – 100 mg magnesium, 3 mg – 8 mg zinc, 30 µg - 250 µg selenium, 1 mg
.25 mg manganese, 2 µg - 75 µg chromium, 2 µg - 75 µg molybdenum, 8 mg – 200 mg
assium, 50 mg – 250 mg citrus fruit peel bioflavanoids, 100 mg – 300 mg arginine, 80 mg
00 mg cysteine, 80 mg – 200 mg inositol, 80 mg – 200 mg carnitine, 3 mg – 35 mg
nzyme Q_{10}, and 3 mg – 35 mg pycnogenol.

st preferably, the recommended amount is 333 µg vitamin A, 3.3 µg vitamin D_3, 3.3 µg
min E, 7 mg vitamin B_1, 7 mg vitamin B_2, 45 mg niacin, 90 µg folic acid, 20 µg vitamin
, 65 µg biotin, 40 mg pantothenic acid, 35 mg calcium, 15 mg phosphorus, 40 mg
gnesium, 7 mg zinc, 20 µg selenium, 1.3 mg manganese, 10 µg chromium, 4 µg
lybdenum, 20 mg potassium, 100 mg citrus fruit peel bioflavanoids, 40 mg arginine, 35 mg
teine, 35 mg inositol, 35 mg carnitine, 7 mg coenzyme Q_{10}, and 7 mg pycnogenol.

ferably, the nutritional composition comprises a daily dosage (based on a human subject of
rage body weight of 72 kg) of 0.9-1,390 µg/kg vitamin A, 0.01-0.694 µg/kg vitamin D_3,
1-0.694 µg/kg vitamin E, 19.4-111 µg/kg vitamin B_1, 19.4-111 µg/kg vitamin B_2, 125-3,472
kg niacin, 0.25-6.94 µg/kg folic acid, 0.05-1.39 µg/kg vitamin B_{12}, 0.181-5.56 µg/kg
tin, 111-1,390 µg/kg pantothenic acid, 97.2-555 µg/kg calcium, 42-4,167 µg/kg
sphorus, 555-2,778 µg/kg magnesium, 6.9-139 µg/kg zinc, 0.28-4.17 µg/kg selenium,
1-208.3 µg/kg manganese, 0.03-2.78 µg/kg chromium, 0.01-1.39 µg/kg molybdenum,
6-4,167 µg/kg potassium, 278-6.944 µg/kg citrus fruit peel bioflavanoids, 139-6,944 µg/kg
inine, 135-5,555 µg/kg cysteine, 69-5,555 µg/kg inositol, 69-5,555 µg/kg carnitine, 22.2-
2 µg/kg coenzyme Q_{10}, and 22.2-972 µg/kg pycnogenol.

re preferably, the nutritional composition comprises a daily dosage (based on a human
ject of average body weight of 72 kg) of 2.31-694 µg/kg vitamin A, 0.023-0.278 µg/kg
amin D_3, 0.023-0.278 µg/kg vitamin E, 48.6-97.2 µg/kg vitamin B_1, 48.6-97.2 µg/kg
amin B_2, 312.5-3,190 µg/kg niacin, 0.6-4.17 µg/kg folic acid, 0.14-0.69 µg/kg vitamin B_{12},
44-4.17 µg/kg biotin, 278-833 µg/kg pantothenic acid, 236-903 µg/kg calcium, 97.2-1,390
/kg phosphorus, 694-1,390 µg/kg magnesium, 41.7-111 µg/kg zinc, 0.42-3.47 µg/kg
enium, 13.9-45.1 µg/kg manganese, 0.07-2.78 µg/kg chromium, 0.03-1.04 µg/kg

molybdenum, 111.1-2,778 μg/kg potassium, 694-3,472 μg/kg citrus fruit peel bioflavanoids, 1,389-4,167 μg/kg arginine, 1,111-2,778 μg/kg cysteine, 1,111-2,778 μg/kg inositol, 1,111-2,778 μg/kg carnitine, 41.7-486 μg/kg coenzyme Q_{10}, and 41.7-486 μg/kg pycnogenol.

Most preferably, the nutritional composition comprises a daily dosage (based on a human subject of average body weight of 72 kg) of 4.6 μg/kg vitamin A, 0.046 μg/kg vitamin D_3, 0.046 μg/kg vitamin E, 97.2 μg/kg vitamin B_1, 97.2 μg/kg vitamin B_2, 625 μg/kg niacin, 1.25 μg/kg folic acid, 0.27 μg/kg vitamin B_{12}, , 0.9 μg/kg biotin, , 555 μg/kg pantothenic acid, 486 μg/kg calcium, 208 μg/kg phosphorus, 555 μg/kg magnesium, 97.2 μg/kg zinc, 0.78 μg/kg selenium, 18.1 μg/kg manganese, 0.14 μg/kg chromium, 0.06 μg/kg molybdenum, 277.8 μg/kg potassium, 1,389 μg/kg citrus fruit peel bioflavanoids, 555 μg/kg arginine, 486 μg/kg cysteine, 486 μg/kg inositol, 486 μg/kg carnitine, 97.2 μg/kg coenzyme Q_{10}, and 97.2 μg/kg pycnogenol. 0.01-0.694 μg/kg vitamin D_3, 18.1 μg/kg manganese, 555 μg/kg arginine, 486 μg/kg cysteine, 4.6 μg/kg vitamin A, 0.046 μg/kg vitamin E, 97.2 μg/kg vitamin B_1, 97.2 μg/kg vitamin B_2, 0.27 μg/kg vitamin B_{12}, 625 μg/kg niacin, 1.25 μg/kg folic acid, 0.9 μg/kg biotin, 555 μg/kg pantothenic acid, 486 μg/kg calcium, 208 μg/kg phosphorus, 555 μg/kg magnesium, 97.2 μg/kg zinc, 0.78 μg/kg selenium, 0.14 μg/kg chromium, 0.06 μg/kg molybdenum, 277.8 μg/kg potassium, 1,389 μg/kg citrus fruit peel bioflavanoids, 486 μg/kg inositol, 486 μg/kg carnitine, 97.2 μg/kg coenzyme Q_{10}, and 97.2 μg/kg pycnogenol.

The present invention provides a method for facilitating bone healing in a mammal, comprising the step of administering to a mammal in need thereof an effective amount of a nutritional composition comprising lysine, proline, ascorbic acid, copper, and vitamin B_6.

The present invention further provides a method for facilitating bone healing in a mammal comprises the step of administering to a mammal in need thereof of an effective amount of a nutritional composition further comprises vitamin A, vitamin D_3, vitamin E, vitamin B_1, vitamin B_2, niacin, folic acid, vitamin B_{12}, biotin, pantothenic acid, calcium, phosphorus, magnesium, zinc, selenium, manganese, chromium, molybdenum, potassium, citrus fruit peel bioflavanoids, arginine, cysteine, inositol, carnitine, coenzyme Q_{10}, and pycnogenol.

Preferably, the mammal is a human.

referably, the nutritional composition is effective in reducing healing time for bone fractures. referably, the healing time is reduced > about 5%. More preferably, the healing time is :duced > about 15%. Most preferably, the healing time is reduced >about 50%.

referably, the nutritional composition is effective in human of all ages. Preferably, the utritional composition is suitable for facilitating bone healing in adults of 41-40 and 41-50 ears of age. The nutritional composition provides a 37 % and 40% reduction in healing time :spectively. More preferably, the nutritional composition is effective in human of 10-20 ears of age (i.e., adolescents), which provides a 49% reduction in healing time.

referably, the nutritional composition may be administered orally, intravenously, or arenterally.

.s used herein, the term "bone healing" refers to the healing of bone fractures. Bone healing nall also encompass the process of bone repair and shall not be limited to healing of :cidental bone fractures. Bone healing also concerns surgical intervention of bones such as one replacement (e.g., hip and knee joint replacement) and bone implantation (e.g., tooth nplantation). When a bone is healed, the normal mobility at the fractured bone site is :stored and there is absence of pain elicited by stressing the fracture or by walking and eneral restoration of efficient and painless functioning of the affected limb at the fracture ite.

he term "healing time" refers to the time elapsed from the time when bone fracture occurs ntil the time when the bone fracture is healed. With respect to the experiments performed in ıe studies disclosed herein, the healing time is measured for the time elapsed from the time f reduction of fractured bone until the bone is healed. "Reduction" refers to the process of ligning the tips of a fractured bone (e.g., tibia) at the point of fracture in a position to allow using of the fractured bone tips together. "Adolescent" is a human between about 10 and bout 20 years of age. "Effective amount" refers to an amount of the present nutritional omposition effective in reducing the healing time of bone fracture. "Pharmaceutically cceptable" refers to carriers, diluents, and excipients that are compatible with the other ngredients of the formulation, and not deleterious to the recipient thereof. "% wt" refers to % f a specific ingredient as a % proportion to the total weight of the nutritional composition,

r example, 27 % wt of lysine refers to a nutritional composition in which 27 % of the total eight of the nutritional composition is lysine.

ıe present nutritional composition is suitable for use in a mammal. Preferably, the mammal a human. Different age groups of human may exhibit different speeds of bone healing. For :ample, human of elder age may be easier to suffer from bone fracture (due to :calcification by osteoporosis) and is likely to have a longer healing time. The present ıtritional composition is found to be effective in faciliating bone healing in human in :neral; and not particularly limited to a particular age group.

he present invention provides a nutritional composition for facilitating bone healing in ıman, preferably in adolescent individuals, comprising the step of administering to a human need of treatment an effective amount of the composition comprising lysine, proline, :corbic acid, copper, and vitamin B_6. Preferably, the nutritional composition contains 27-34 ı wt lysine, 42-47 % wt ascorbic acid and 14-15 % wt proline.

he present nutritional composition also contains lysine and proline. Lysine and proline are ɔnstituents of collagen and proteins in the bone. Lysine and proline may contribute to ;teoblast proliferation of alkaline phosphatase, nitric oxide, insulin like growth factor-I, and ɔllagen type I and may be essential for proper bone formation.

ysine may include lysine salts such as hydroxylysine and hydroxylysine salts. A daily dose f 3.2 - 139 mg/kg lysine is recommended. Preferably, 14 to 111 mg/kg lysine is used; and ıore preferably, 14 mg/kg lysine is used. For an average individual weighing 72 kg, the daily :commended dosage of lysine is 230 mg to 10 grams; preferably, 1,010 mg to 8 grams; and ıore preferably 1,010 mg.

roline is a non-essential amino acid. However, its synthesis in human body could be limited nder certain conditions. It has been reported that the stress of fracture lowers non-essential mino acid levels in plasma of elder humans. In such a case, deficiency of proline, a semi-ssential amino acid, if any, would adversely affect the healing of fracture, since this amino :id is present in a large proportion in collagen.

roline may include proline salts such as hydroxyproline and hydroxyproline salts. A daily ose of 1.7- 69.4 mg/kg proline is recommended. Preferably, 7.8 to 56 mg/kg is used; and

nore preferably, 7.8 mg/kg is used. For an average individual weighing 72 kg, the daily ecommended dosage of proline is 120 mg to 5 grams; preferably, 560 mg to 4 grams; and nore preferably 560 mg.

The present nutritional composition contains ascorbic acid. Ascorbic acid may promote the rogressive development of osteoblast phenotype and facilitate bone healing and it is also ecessary for the differentiation and proliferation of osteogenic and chondrogenic cells.

Ascorbic acid and vitamin C are used interchangeably. The term ascorbic acid encompasses scorbic acid and salts thereof. Preferably, the ascorbic acid to be applied in accordance with he present invention is calcium ascorbate, magnesium ascorbate or ascorbyl palmitate. A laily dose of 5 - 208 mg/kg ascorbic acid is recommended. Preferably, 20.8 - 125 mg/kg is sed; and more preferably, 20.8 mg/kg is used. For an average individual weighing 72 kg, the laily recommended dosage of ascorbic acid is 360 mg to 15 grams; preferably, 1,500 mg to 9 grams; and more preferably 1,500 mg.

The present invention further provides a nutrient composition further comprising minerals nd/or trace elements. Trace elements may help to catalyze the production of these nacromolecules needed for connective tissue structure and function. Preferred trace elements n accordance with the present invention are iron, iodine, copper, zinc, manganese, cobalt, nolybdenum, selenium, chromium, nickel, tin, fluorine or vanadium.

Copper, as a cofactor for lysyl oxidase, is essential for intra- and intermolecular cross-links in collagen. Copper deficit has been shown to impair the mechanical strength of bone.
It was hypothesized that a relatively large quantity of ascorbic acid, vitamin B_6, L-lysine and L-proline, together with copper, would have a pronounced effect on bone collagen health and function to produce a marked difference in healing time between fractured bones of a supplement group and a placebo group.

Copper compounds may include copper glycinate. A daily dose of 0.02 to 278 µg/kg copper is recommended. Preferably, 0.03 to 83 µg/kg is used; and more preferably, 4.6 µg/kg is used. For an average individual weighing 72 kg, the daily recommended dosage of 1.5 µg to 20 mg; preferably 2 µg to 6 mg; and more preferably, 330 µg.

Vitamin B_6 is of importance in bone healing, as it is instrumental in providing reducing equivalents necessary for mineralization. Vitamin B_6 deficiency caused marked diminution in glucose 6-phosphate dehydrogenase activity in perisoteal bone formation and in developing callus. It also caused changes in the bone suggestive of imbalance between osteoblasts and osteoclasts.

Vitamin B_6 compounds may include pryridosine HCl. A daily dose of 2.8 to 279 µg/kg vitamin B_6 is recommended. Preferably, 7 to 139 µg/kg vitamin B_6 is used; and more preferably, 139 µg/kg is used. For an average individual weighing 72 kg, the daily recommended dosage of vitamin B_6 is 0.2 to 20 mg; preferably, 0.5 to 10 mg; and more preferably, 10 mg.

Certain ingredients of the nutritional composition according to the invention are present at a high amount. Specifically, lysine is present between 27-34 % wt (preferably at 28 - 33 % wt); proline is present between 14 – 16 % wt (preferably 15-16 % wt); and ascorbic acid is present between 42-47 % wt (preferably at 43-46 % wt).

Unexpectedly, it has been found in accordance with the present invention that a nutritional composition as specified herein efficiently facilitates bone healing (i.e. it reduces the healing time) of bone fractures in humans, particularly in adolescents. Advantageously, administration of the nutritional composition of the present invention does not merely facilitate bone healing efficiently, but also improves general well being, and is cost effective.

A recommended daily oral dosage includes 3.2-139 mg/kg lysine, 1.37-69 mg/kg proline, 5-208 mg/kg ascorbic acid, 2.78-279 µg/kg vitamin B_6, and 0.02-278 µg/kg copper. Preferably, the recommended daily oral dosage is: 14-111 mg/kg lysine, 7.8 - 56 mg/kg proline, 20.8-125 mg/kg ascorbic acid, 6.9-139 µg/kg vitamin B_6, and 0.03-83 µg/kg copper. More preferably, the recommended daily oral dosage is: 14 mg/kg lysine, 7.8 mg/kg proline, 20.8 mg/kg ascorbic acid, 139 µg/kg vitamin B_6, 4.6 µg/kg copper. Preferably, the nutritional composition is administered 3 tablets per day (i.e., one tablet in morning, one tablet in afternoon and one tablet at night).

Several other dietary components, such as: protein, calcium, magnesium, zinc, copper, iron, fluoride, and vitamins D, A and K, are required for normal bone metabolism. All of these nutrients impact fracture healing, some more directly than others. The trauma of bone

fracture was shown to cause a decrease in copper, manganese, and zinc levels in liver, suggesting increased requirement of these minerals after bone fracture.

The present nutritional composition may further comprise vitamin D_3, manganese, arginine, cysteine, vitamins A, E, B_1, B_2, B_{12}, niacin, folic acid, biotin, pantothenic acid, calcium, phosphorus, magnesium, zinc, selenium, chromium, molybdenum, potassium, citrus fruit peel bioflavanoids, inositol, carnitine, coenzyme Q_{10}, and pycnogenol.

The particular dosage of the present nutritional composition required to facilitate bone healing (i.e., reducing healing time) will depend on the severity of the medical condition, the route of administration and the particular subject being treated. The nutritional composition of the present invention may be administered by a variety of routes including oral, intravenous, or parenteral administration. Preferably, the nutritional composition is in unit dosage form, e.g. tablets or capsules. Thus, the present nutritional composition is recommended to be administered as an orally tablet preparation.

A daily recommended dosage (based on a human subject of average body weight of 72 kg) may further contain 0.9-1,390 μg/kg vitamin A, 0.01-0.694 μg/kg vitamin D_3, 0.01-0.694 μg/kg vitamin E, 19.4-111 μg/kg vitamin B_1, 19.4-111 μg/kg vitamin B_2, 125-3,472 μg/kg niacin, 0.25-6.94 μg/kg folic acid, 0.05-1.39 μg/kg vitamin B_{12}, 0.181-5.56 μg/kg biotin, 111-1,390 μg/kg pantothenic acid, 97.2-555 μg/kg calcium, 42-4,167 μg/kg phosphorus, 555-2,778 μg/kg magnesium, 6.9-139 μg/kg zinc, 0.28-4.17 μg/kg selenium, 11.1-208.3 μg/kg manganese, 0.03-2.78 μg/kg chromium, 0.01-1.39 μg/kg molybdenum, 55.6-4,167 μg/kg potassium, 278-6.944 μg/kg citrus fruit peel bioflavanoids, 139-6,944 μg/kg arginine, 135-5,555 μg/kg cysteine, 69-5,555 μg/kg inositol, 69-5,555 μg/kg carnitine, 22.2-972 μg/kg coenzyme Q_{10}, and 22.2-972 μg/kg pycnogenol.

Preferably, the daily recommended dosage (based on a human subject of average body weight of 72 kg) may further contain 2.31-694 μg/kg vitamin A, 0.023-0.278 μg/kg vitamin D_3, 0.023-0.278 μg/kg vitamin E, 48.6-97.2 μg/kg vitamin B_1, 48.6-97.2 μg/kg vitamin B_2, 312.5-3,190 μg/kg niacin, 0.6-4.17 μg/kg folic acid, 0.14-0.69 μg/kg vitamin B_{12}, 0.444-4.17 μg/kg biotin, 278-833 μg/kg pantothenic acid, 236-903 μg/kg calcium, 97.2-1,390 μg/kg phosphorus, 694-1,390 μg/kg magnesium, 41.7-111 μg/kg zinc, 0.42-3.47 μg/kg selenium, 13.9-45.1 μg/kg manganese, 0.07-2.78 μg/kg chromium, 0.03-1.04 μg/kg molybdenum, 111.1-2,778 μg/kg potassium, 694-3,472 μg/kg citrus fruit peel bioflavanoids, 1,389-4,167

g/kg arginine, 1,111-2,778 μg/kg cysteine, 1,111-2,778 μg/kg inositol, 1,111-2,778 μg/kg rnitine, 41.7-486 μg/kg coenzyme Q_{10}, and 41.7-486 μg/kg pycnogenol.

lost preferably, the nutritional composition may further include a daily dosage (based on a uman subject of average body weight of 72 kg) of 4.6 μg/kg vitamin A, 0.046 μg/kg vitamin '3, 0.046 μg/kg vitamin E, 97.2 μg/kg vitamin B_1, 97.2 μg/kg vitamin B_2, 625 μg/kg niacin, .25 μg/kg folic acid, 0.27 μg/kg vitamin B_{12}, , 0.9 μg/kg biotin, , 555 μg/kg pantothenic acid, 86 μg/kg calcium, 208 μg/kg phosphorus, 555 μg/kg magnesium, 97.2 μg/kg zinc, 0.78 g/kg selenium, 18.1 μg/kg manganese, 0.14 μg/kg chromium, 0.06 μg/kg molybdenum, 77.8 μg/kg potassium, 1,389 μg/kg citrus fruit peel bioflavanoids, 555 μg/kg arginine, 486 g/kg cysteine, 486 μg/kg inositol, 486 μg/kg carnitine, 97.2 μg/kg coenzyme Q_{10}, and 97.2 g/kg pycnogenol. 0.01-0.694 μg/kg vitamin D_3, 18.1 μg/kg manganese, 555 μg/kg arginine, 86 μg/kg cysteine, 4.6 μg/kg vitamin A, 0.046 μg/kg vitamin E, 97.2 μg/kg vitamin B_1, 97.2 g/kg vitamin B_2, 0.27 μg/kg vitamin B_{12}, 625 μg/kg niacin, 1.25 μg/kg folic acid, 0.9 μg/kg iotin, 555 μg/kg pantothenic acid, 486 μg/kg calcium, 208 μg/kg phosphorus, 555 μg/kg iagnesium, 97.2 μg/kg zinc, 0.78 μg/kg selenium, 0.14 μg/kg chromium, 0.06 μg/kg iolybdenum, 277.8 μg/kg potassium, 1,389 μg/kg citrus fruit peel bioflavanoids, 486 μg/kg iositol, 486 μg/kg carnitine, 97.2 μg/kg coenzyme Q_{10}, and 97.2 μg/kg pycnogenol.

he present nutritional composition may include a pharmaceutically acceptable carrier, iluent, or excipient. Nutritional composition of the present invention can be prepared by rocedures known in the art. Respective ingredients may be formulated with common xcipients, diluents, or carriers, and formed into tablets, capsules, suspensions, powders, and ie like. Examples of excipients, diluents, and carriers include: i) fillers and extenders such as tarch, sugars, mannitol, and silicic derivatives; ii) binding agents such as carboxymethyl ellulose and other cellulose derivatives, alginates, gelatin, and polyvinyl-pyrrolidone; iii) ioisturizing agents such as glycerol; disintegrating agents such as calcium carbonate and odium bicarbonate; agents for retarding dissolution such as paraffin; iv) resorption ccelerators such as quaternary ammonium compounds; v) surface active agents such as cetyl alcohol, and glycerol monostearate; v) adsorptive carriers such as kaolin and bentonite; nd vi) lubricants such as talc, calcium and magnesium stearate, and solid polyethyl glycols.

he nutritional compositions may also be formulated as elixirs or solutions for convenient ral administration or as solutions appropriate for parenteral administration, for example, by ntramuscular, subcutaneous or intravenous routes. Ideally the formulation is in the form of a

pill, tablet, capsule, lozenge, liquid or similar dosage form. The nutritional compositions may well be suited to formulation as sustained release dosage forms and the like.

Tablets Preparation

The ingredients listed in Table 1 were formulated to form tablets. The tablets contained the key ingredients of lysine (1,010 mg), proline (560 mg), ascorbic acid (1,500 mg), copper (330 µg) and vitamin B_6 (10 mg).

The tablets further contained additional ingredients including vitamin D_3, manganese, arginine, cysteine, vitamins A, E, B_1, B_2, B_{12}, niacin, folic acid, biotin, pantothenic acid, calcium, phosphorus, magnesium, zinc, selenium, chromium, molybdenum, potassium, citrus fruit peel bioflavanoids, inositol, carnitine, coenzyme Q_{10}, and pycnogenol.

Table 1 - Serving Size - Three Tablets/Day

Key Ingredients	**Daily Dosage**	**Daily Dosage per Body Weight***
L-Lysine (from L-Lysine HCl)	1,010 mg (28 % wt)	14.0 mg/kg
L-Proline	560 mg (16 % wt)	7.8 mg/kg
Ascorbic Acid (Ascorbyl Palmitate, Calcium Ascorbate, Magnesium Ascorbate)	1,500 mg (42.8 % wt)	20.8 mg/kg
Copper (Copper Glycinate)	330 µg (<0.01 % wt)	4.58 µg/kg
Vitamin B_6 (Pyridoxine HCl)	10 mg (0.28 % wt)	139 µg/kg
Additional Ingredients		
Vitamin A (7.5% Betatene (Henkel))	333 µg	4.6 µg/kg
Vitamin D_3 (Cholecalciferol)	3.3 µg	0.046 µg/kg
Vitamin E (Mixed Covitol)	3.3 µg	0.046 µg/kg
Vitamin B_1 (Thiamine Mononitrate)	7 mg	97.2 µg/kg
Vitamin B_2 (Riboflavin)	7 mg	97.2 µg/kg
Niacin (Niacinamide)	45 mg	625 µg/kg
Folic Acid	90 µg	1.25 µg/kg
Vitamin B_{12} (Cyanocobalamin)	20 µg	0.27 µg/kg
Biotin	65 µg	0.90 µg/kg
Pantothenic Acid (D-Calcium Pantothenate)	40 mg	555 µg/kg
Calcium (Gycinate, Ascorbate)	35 mg	486 µg/kg
Phosphorus (Dicalcium Phosphate)	15 mg	208 µg/kg
Magnesium (Magnesium Glycinate, Magnesium Ascorbate)	40 mg	555 µg/kg

inc (Zinc Glycinate)	7 mg	97.2 µg/kg
elenium (L-Selenomethionine)	20 µg	0.78 µg/kg
anganese (Amino Acid Chelate)	1.3 mg	18.1 µg/kg
hromium (Chromium Glycanate)	10 µg	0.14 µg/kg
olybdenum (Molybdenum Glycinate)	4 µg	0.06 µg/kg
otassium (Potassium Proteinate)	20 mg	277.8 µg/kg
itrus Fruit Peel Bioflavanoids	100 mg	1,389 µg/kg
-Arginine (L-Arginine HCl)	40 mg	555 µg/kg
-Cysteine (L-Cysteine Monohydrate ICl)	35 mg	486 µg/kg
nositol	35 mg	486 µg/kg
-Carnitine (L-Carnitine Tartrate)	35 mg	486 µg/kg
oEnzyme Q_{10}	7 mg	97.2 µg/kg
ycnogenol	7 mg	97.2 µg/kg
OTAL	**3.5 g**	**48.6 mg/kg**

ody Weight refers to a human subject of average body weight of 72 kg

inical Studies

tient Selection:

randomized double-blind placebo-controlled study was performed. The clinical study was nducted according to the recommendations of the Declaration of Helsinki, its amendments d AMG. The inclusion criteria for patient admission were: (i) unilateral displaced closed or ade I open fractures of tibial shaft; and (ii) age above 10 years. The exclusion criteria for tient admission were: (i) patients who had other major injuries, (ii) patients with rdiopulmonary, rheumatological, neurological or metabolic diseases, (iii) patients with evious injuries which influenced their general functions, (iv) patients with fractures within 5 n distal to tibial tuberosity or within 5 cm proximal to the ankle joint.

dmitted patients were either receive standard care and placebo or standard care with pplementation with a nutritional supplement comprising lysine, proline, ascorbic acid, pper and vitamin B_6. Qualifying patients, on admssion to the study, were clinically amined and the radiographs of the affected limbs were taken, fractures reduced under esthesia and above knee plaster casts applied.

fforts were made to ensure that age groups and fracture types were equally distributed in the o groups. Patients were entered in the study from February 2001 until December 2002. A tal of 113 patients with unilateral displaced closed or grade I open tibial fractures studied. dmitted patients were given informed consent. Participants were advised that data obtained om the studies would be submitted for publication. Out of these, 54 patients were assigned

) the supplemented group and 59 pateients were assigned to the placebo group. Table 2 ummarizes the age distribution of the total 131 patients.

'able 2 - Distribution of Patients by Age Groups

Age	10 –20 years	21-30 years	31-40 years	41-50 years	> 50 years	Total
Supplemented Group	2 (9.5%)*	7 (33.3%)	6 (28.6%)	3 (14.3%)	3 (14.3%)	21 (100%)
Placebo Group	8 (22.2%)	10 (27.8%)	10 (27.8)%	4 (11.1%)	4 (11.1%)	36 (100%)

Values in parenthesis represent percentage of total patients in the specified age group

'linical Protocol:

ll fractures were reduced (closed reduction) under anesthesia and above knee plaster casts pplied. The fractured limbs were routinely radiographed before and after reduction. The upplemented group of patients were supplied with the nutritional composition in Table 1 and ie placebo group of patients with bottles containing placebo tablets. The nutritional omposition listed in Table 1 were studied to evaluate if the proposed desired supplements an ensure adequacy of nutrients impacting fracture healing. The placebo tablets contained iaterial of no medical significance, such as cellulose, fructose etc., but were physically idistinguishable from nutrient tablets. All patients were asked to take one tablet thrice daily norning, afternoon and night) .

Jrine samples of the patients were taken to evaluate their baseline ascorbic acid levels. Blood amples were also taken to assess the baseline calcium levels (Figure 3). The patients were ien discharged from the hospital and were asked to return for check-up every four weeks ntil the treating orthopedic surgeon deemed the fracture healed. At each follow-up xamiantion, tibial fractures were radiographed, and patients were clinically examined and ested for urinary vitamin C and blood calcium levels. The radiological examination was done o confirm that the fragments of the fracture remained in reduced position and that callus ormation was progressing satisfactorily. The ascorbic acid content of the urine was etermined by spectrophotometry and the blood calcium content was determined with ommercial kits. Healing was defined as absence of abnormal mobility at the fracture site linically and absence of pain elicited by stressing the fracture or by walking. Radiographic onfirmation of callus formation was used as supporting evidence for healing. (See Figures 1

d 2 for radiographic examples). A healing period of greater than 20 weeks without any rgical intervention was considered delayed healing.

atistical Analysis

ıe results were expressed as means ± standard error for the groups. The Wilcoxon test was rformed on the fracture healing times among various groups. Statistical significance was : at $P < 0.05$.

:sults:

patients from the supplemented group and 42 patients from the placebo group returned for gular follow-ups until fractures were deemed healed.

/erall, the urinary ascorbic acid content in supplemented patient group was higher than that the placebo group (Figure 3). Low urinary ascorbic acid values (below 5 mg/100 ml of ine) were detected in eight supplemented patients and high ascorbic acid values (above 5 g/100 ml of urine) in six placebo patients at any one of the check-up visits. These patients :re excluded from evaluation. Therefore, only 21 patients in supplemented group and 36 tients in the placebo group remained for the completion of the study. Blood calcium levels all participants in the study were within normal limits.

ıe age distribution of the patients is detailed in Table 2. The age range of the patients in the pplemented group was between 15 to 65 years, with a mean age of 35 years. The age range the patients in the placebo group was between 12 to 75 years, with a mean age of 32 years.

ıe healing time of the patients is detailed in Table 4. The mean healing time for the patients the supplemented group was 14.0 ± 1.1 weeks. The mean healing time for the patients in the acebo group was 16.9 ± 1.2 weeks. These data show that the overall mean healing time in e supplemented group is about three weeks shorter than that for the placebo group (i.e., 17.2). This difference attain statistical significance at $t=1.07$, $p=0.288$.

:rcentile classification of the data indicates that in the 75th percentile, fractures in the pplemented group healed within 17 weeks, while those in placebo group healed in 19 weeks 'able 3).

Table 3 - Effect of Supplementation on Bone Fracture Healing Time

Criteria	Supplemented Group	Placebo Group
Number of patients	21	36
Age range (years)	15 to 65	12 to 75
Mean age (years)	35	32
Healing time (weeks)	14.0±1.1	16.9±1.2
75th percentile healing period (weeks)	17	19

The percentage of patients experiencing early fracture healing (in 10 weeks or less) differed by 33.3% in the supplemented group and 11.1% in the placebo group (Chi-square analysis=2.853, p=0.091). The percentage of patients that had delayed healing (more than 20 weeks) was 9.5% in supplemented group and 19.4 % in the placebo group (Table 4).

Table 4 - Distribution (Percentage) of Patients by Fracture Healing Time

Healing Time	Supplemented Group	Placebo Group
10 weeks or less	33.3%	11.1%
11 to 15 weeks	33.3%	52.8%
16 to 20 weeks	23.8%	16.7%
More than 20 weeks	9.5%	19.4%

The healing time of bone fracture in patients of different ages is shown in Table 6. In the patients of 10-20 years of age, the healing time reduced from 17.6 weeks to 9 weeks (reduced 49 %). In the patients of 31-40 years of age, the healing time reduced from 17.1 weeks to 10.7 weeks (reduced 37%). In the patients of 41-50 years of age, the healing time reduced from 21.2 weeks to 12.7 weeks (reduced 40%). In the patients of >50 years of age, the

aling time reduced from 16 weeks to 15.7 weeks (reduced 1.8%). Overall, the healing time the supplemented group, except in the 21-30 year old group, is reduced. (Table 5).

ble 5 - Healing Time (in Weeks) for Various Age Groups

e Groups	10 -20 yr	21-30 yr	31-40 yr	41-50 yr	> 50 yr
pplemented Group					
aling Time					
an (in wks)	9	18.3	10.7	12.7	15.7
nge (in wks)	9	9-26	6-16	11-14	14-19
icebo Group					
aling Time					
an (in wks)	17.6	14.6	17.1	21.2	16
nge (in wks)	9-30	9-30	11-39	13-30	10-20

e data suggest that administering to patients suffering from bone fracture with the present tritional composition at the specified doses effectively reduces the healing time by at least o weeks in 75% of the patients. Patients in the supplemented group also reported an hanced feeling of general well being during the study. The strongest effects can be seen in adolescent age group (i.e., 10 to 20 years of age) who has a 49% reduction in the healing ne. Patients who are 41-50 years and 31-40 years of age also have a significant reduction in healing time (i.e., 40 % and 37%, respectively). It is believed that patients in the other age oups may likely to receive the same benefits if the dosage of the nutritional supplementation optimized.

ere was no signficantly difference in the blood calcium levels in the supplemented group as mpared to that in the placebo group. All the patients were found to have their blood lcium levels within the normal range.

e reduction in healing time is believed to be due to the supplementation of key ingredients hich comprise lysine, proline, ascorbic acid, copper and vitamin B_6, as well as additional gredients used in the present study. The present data provide evidence that nutritional pplementation in patients suffering from bone fracture can facilitate the healing (i.e.,

:duce healing time). Administeration of the present nutritional compositions to bone :actured patients would have a positive impact early functional recovery, improved well :ing, reduced medical costs, and reduced cost to business.

will be understood that there is no intent to limit the present invention to the preferred nbodiment disclosed, but rather it is intended to cover all modifications and alternate onstructions falling within the spirit and scope of the invention. All publications and other :ferences mentioned herein are incorporated by reference in their entirety.

'he figures show:

igure 1 depicts a radiograph of tibial shaft fracture immediately prior to reduction.

igure 2 depicts radiograph of tibial shaft fracture at healing at 12 weeks.

igure 3 depicts ascorbic acid levels (urinalysis) in supplemented (patient #1-29) and placebo atient #30-70) groups.

igure 4 depicts a distribution of patients (percentage) by tibial fracture healing time.